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Muscimol, a Psychoactive Constituent of *Amanita Muscaria*, as a Medicinal Chemical Model Structure

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The mushroom *Amanita muscaria* Fr., the fly agaric, has powerful psychotropic effects, which manifest themselves after consumption of the fresh or dried mushrooms.[1] The use of the fly agaric as an inebriant among certain Siberian tribes may represent a degeneration of traditions, which played a decisive part in ancient Indo-European religious ceremonies.[2] The plant Soma, which was deified and consumed by the Indo-European priests, probably is identical with *Amanita muscaria* Fr.[3]

During the past century great efforts have been made to isolate and identify the psychoactive constituents of *Amanita muscaria* Fr. In 1954 muscarine chloride [(2*S*,4*R*,5*S*)-(+)-2-trimethylammoniummethyl-4-hydroxy-5-methyltetrahydrofuran chloride] was finally isolated from the fly agaric in a pure state and its structure unequivocally established.[4,5] While muscarine has powerful cholinergic effects on the peripheral nervous system, the quaternary ammonium group prevents muscarine from entering the central nervous system (CNS) after peripheral administration. Thus, muscarine cannot be responsible for the central effects of the fly agaric.

Ten years later three groups independently reported the isolation and structure determination of centrally active constituents of *Amanita muscaria* Fr.[6–8] The compounds proved to be 3-isoxazolol derivatives with zwitterionic structures. Ibotenic acid [(*RS*)-α-amino-3-hydroxyisoxazol-5-ylacetic acid hydrate] is synthesized by the mushroom, whereas muscimol (5-aminomethyl-3-isoxazolol) (Fig. 1) appears to be formed in the plant material by

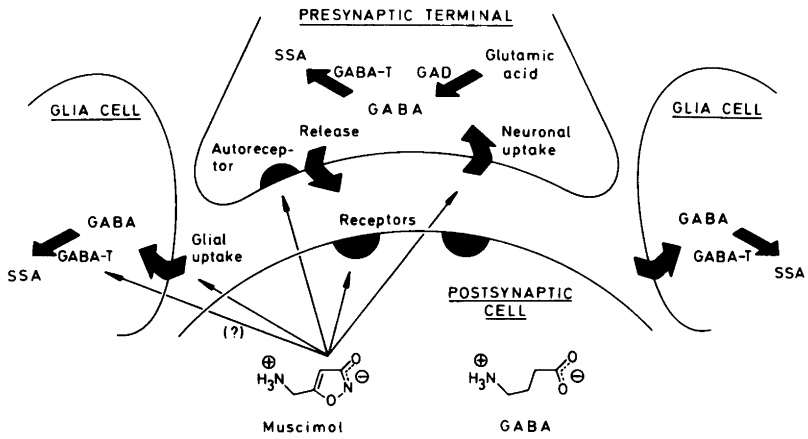

Fig. 1. An illustration of the processes and functions in a central GABA-mediated synapse and of the sites of attack of muscimol. GAD is glutamic acid decarboxylase, GABA-T is GABA:2-oxoglutarate aminotransferase, and SSA is succinic semialdehyde.

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decarboxylation of ibotenic acid, a process which proceeds very easily and without enzyme catalysis.[5]

Muscimol, which is a structural analogue of GABA (γ-aminobutyric acid), interferes with the central inhibitory GABA neurotransmitter system (Fig. 1). Muscimol shows *in vitro* a very high affinity for the postsynaptic GABA receptors[9,10] and also for the presynaptic autoreceptors assumed to regulate the synaptic release of GABA.[11,12] *In vivo* muscimol is capable of activating the GABA receptors in a manner similar to that of GABA itself.[13,14] Furthermore, muscimol interacts with the GABA transport (uptake) systems,[15] being a substrate for the neuronal[16] and possibly also for the glial transport carriers.[15] Although muscimol is not a substrate for the GABA-metabolizing enzyme GABA:2-oxoglutarate aminotransferase (GABA-T) *in vitro*,[17] it is very rapidly decomposed *in vivo* probably by a transamination reaction involving the aminomethyl side chain.[18,19] The metabolites of muscimol have not yet been identified, but such products formed intracellularly may contribute to the pronounced toxicity of muscimol.[20] These multiple effects of muscimol at GABA synapses, which obviously limit its utility as a pharmacological tool, probably are the cause of the psychoactive properties of muscimol.

In contrast to GABA[21] muscimol can, in spite of its zwitterionic structure, penetrate the blood-brain barrier (BBB).[11,19] This ability and the restrained conformational mobility of the muscimol molecule form the basis of our extensive use of muscimol as a model structure for the development of compounds with specific actions on the GABA receptors (GABA agonists) and of inhibitors of the GABA uptake processes.[22–26] Such compounds with adequate pharmacokinetic properties have considerable therapeutical interest, as decreased function, and in some cases partial degeneration of central GABA neurones, appear to contribute to the development of severe diseases like Huntington's chorea, parkinsonism, schizophrenia, and epilepsy[28] (for references, see also 28 – 32).

BIOLOGICAL AND BIOCHEMICAL TEST SYSTEMS

The GABA agonist activity of the GABA and muscimol analogues, that is their ability to activate the postsynaptic GABA receptors *in vivo* (intrinsic activity) with subsequent depression of the firing of the nerve cells on which the receptors are located, has been determined microelectrophoretically. Using this technique[13,33,34] the compounds have been administered on living cells in the cat spinal cord. The depressant effects of the compounds, and the sensitivity of these effects to the GABA antagonist bicuculline methochloride (BMC) have been compared with those of GABA itself. Thus, a GABA agonist is defined as a BMC-sensitive depressant of neuronal firing.[35]

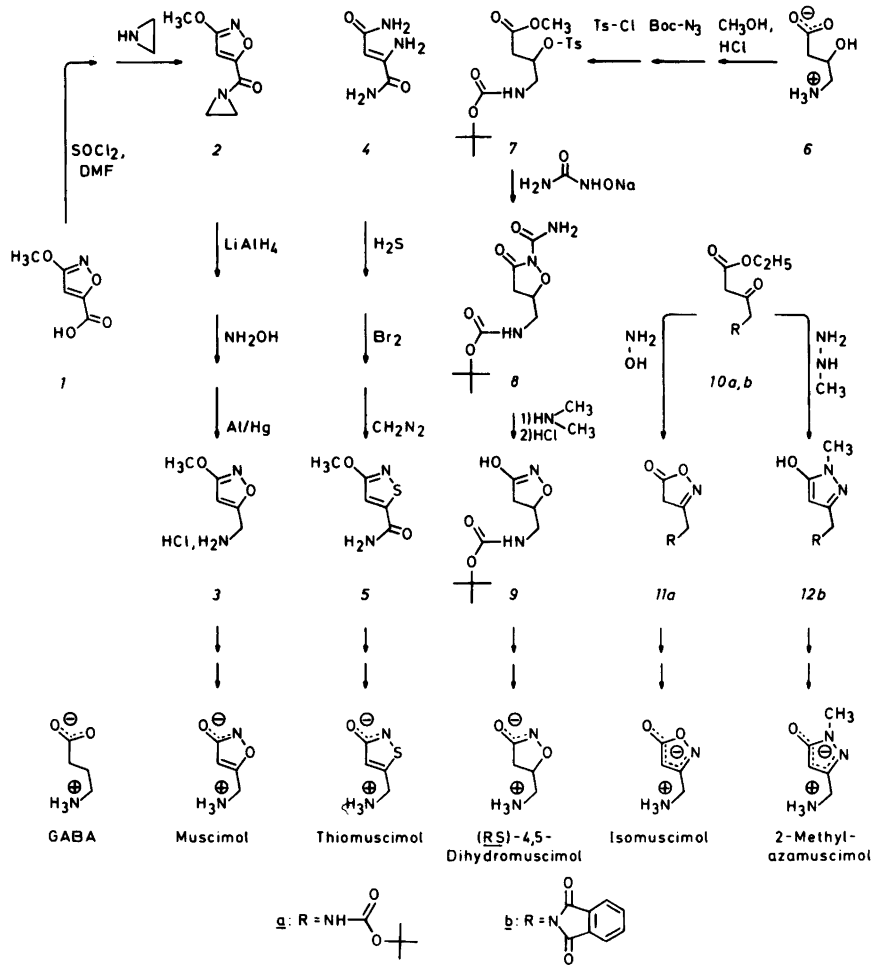
The affinity of the compounds for GABA receptor sites on rat brain membranes *in vitro* has been measured using a modified version[10] of the radiochemical method described by Enna and Snyder (1975).[36] The ability of the compounds to displace radioactive GABA from the receptor sites are expressed as IC_{50} values (the concentrations of the compounds required for 50% displacement of the radioactive ligand).

Earlier described methods have been used to study the effects of the compounds on the neuronal GABA uptake system *in vitro*. In these studies the capacity of the compounds for preventing mini-slices of mouse[15] or rat[37] brains from accumulating radioactive GABA is determined. The inhibitory effects of the compounds on glial GABA uptake have been measured analogously using cultured astrocyte cells.[15] The degree of inhibition of the GABA uptake processes by the compounds is expressed as IC_{50} values (the concentrations of inhibitor required for 50% reduction of the capacities of these brain tissue preparations for accumulating radioative GABA).

DESIGN AND DEVELOPMENT OF SPECIFIC GABA AGONISTS

Monocyclic muscimol analogues

The GABA molecule has considerable flexibility as a result of free rotation around single bonds (Fig. 1). This conformational mobility is reduced in the GABA analogue muscimol (Fig. 1), in which the 3-isoxazolol moiety acts as a masked carboxyl group and restricts conformation. The affinity of muscimol for the GABA receptors *in vitro* (IC_{50} 0.006 μM) is higher than that of GABA (IC_{50} 0.03 μM),[10] and furthermore muscimol is a more powerful GABA agonist *in vivo* than GABA.[10,13] These properties of muscimol have prompted us to synthesize a series of analogues containing different 5-membered heterocyclic rings with acidic hydroxy groups and aminomethyl side chains in positions



Scheme 1.

equivalent to those in muscimol. The syntheses of some of these analogues are outlined in Scheme 1.

The key steps in the synthesis of muscimol [38] are conversion of *1* into its acid chloride and reduction of *2* to the corresponding aldehyde. The former reaction required drastic conditions, and in contrast to general findings for analogous reactions satisfactory yields of the aldehyde intermediate could only be obtained using equimolar amounts of *2* and lithium aluminium hydride.

Treatment of *4* with hydrogen sulfide gave the dimerized product dithiosuccinamide and not the expected thioenol or 2,2-dithiol.[39] Nevertheless, oxidation of the dimerized intermediate with bromine gave 3-hydroxyisothiazole-5-carboxamide. By treatment with diazomethane this was converted into a separable mixture of *5* and the corresponding *N*-methylated product 2-methyl-3-oxo-4-isothiazoline-5-carboxamide. Reduction of *5* and subsequent deprotection gave thiomuscimol.[39]

The critical step in the reaction sequence for the preparation of (*RS*)-4,5-dihydromuscimol is the cyclization reaction between the protected 3-OH-GABA derivative *7* and hydroxyurea sodium salt.[40] Under certain conditions a separable mixture of *8* and *9* was obtained.[40] Under slightly different conditions *8* was obtained as the only product, and *8* could be converted into *9* by treatment with dimethylamine (J. S. Johansen and P. Krogsgaard-Larsen, unpublished), which traps the isocyanate ion formed by decomposition of the amide group.

Isomuscimol and 2-methylazamuscimol were

synthesized *via* cyclization of the appropriate β-oxoesters *10a,b* with hydroxylamine and methylhydrazine, respectively.[41]

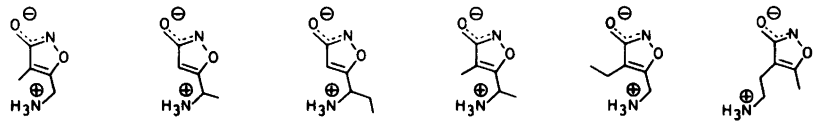
In spite of an immediate structural similarity of these muscimol analogues (Scheme 1), the activities of the compounds are strictly dependent on the structure of the heterocyclic rings.[10,23,25] While the receptor affinities of (*RS*)-4,5-dihydromuscimol (IC_{50} 0.009 μM) and thiomuscimol (0.02 μM) are similar to that of muscimol (IC_{50} 0.006 μM), isomuscimol (IC_{50} 29 μM) and 2-methylazamuscimol ($IC_{50} > 100$ μM) bind very poorly to GABA receptor sites *in vitro*.[10] Accordingly, (*RS*)-4,5-dihydromuscimol and thiomuscimol, like muscimol, are more potent than GABA itself as GABA agonists *in vivo*, whereas isomuscimol is very weak.[10] 2-Methylazamuscimol has not been tested for GABA agonist activity *in vivo*.

The hydroxylated ring systems in these compounds have quite different protolytic properties. The pK_A values for these compounds and GABA are: GABA (4.0; 10.7),[10] muscimol (4.8; 8.4),[10] thiomuscimol (6.1; 8.9),[39] (*RS*)-4,5-dihydromuscimol (5.8; 9.3)[40] and isomuscimol (2.6; 9.0).[41] There is, however, no simple relationship between the pK_A values of these zwitterions and their GABA agonist activities. The degree of delocalization of the negative charges of these compounds apparently is a factor of importance for their interaction with the GABA receptors.[10] Based on X-ray crystallographic studies the charge of the 5-isoxazolol anion of isomuscimol[42] is highly delocalized (Scheme 1), whereas that of muscimol is only slightly delocalized in the ring[43] in agreement with their respective pK_A I values. Thus, the charge distribution of muscimol and to a still larger extent that of (*RS*)-4,5-dihydromuscimol, both of which are potent GABA agonists, seem to be similar to that determined for GABA.[44] UV spectroscopic studies indicate that the negative charges of isomuscimol and 2-methylazamuscimol are delocalized to approximately the same extent.[41] The charge distribution of thiomuscimol has not been studied, but thiomuscimol is assumed to resemble muscimol (Scheme 1).

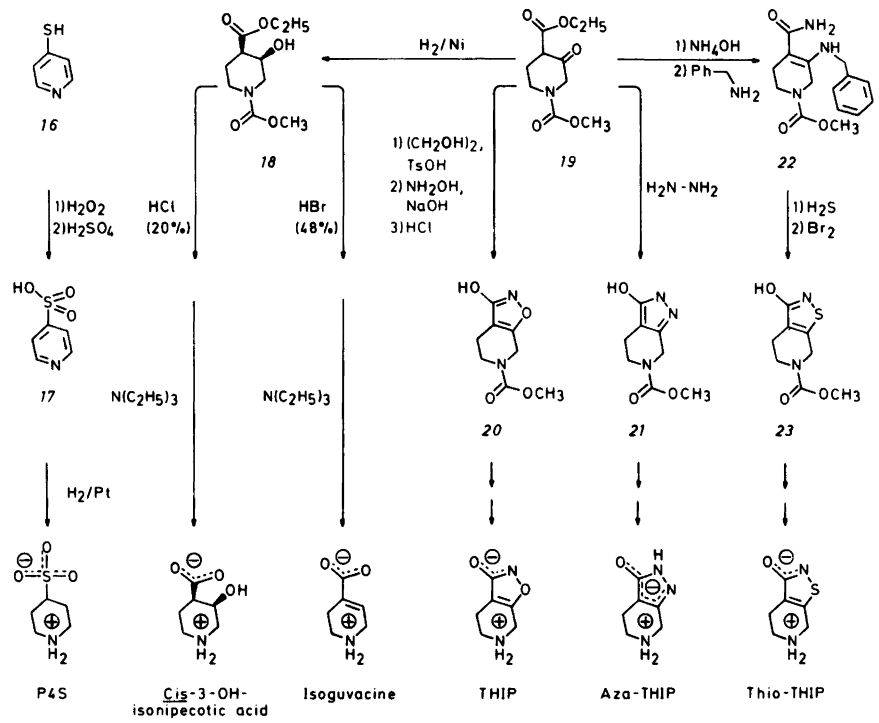
Although thiomuscimol and (*RS*)-4,5-dihydromuscimol do not affect significantly the activity of GABA-T *in vitro*,[10] the aminomethyl side chains of these compounds may be just as susceptible to metabolic degradation as that of muscimol. Therefore these muscimol analogues have only been studied pharmacologically to a very limited extent.[45]

The structural constraints imposed on agonists for the GABA receptors have been further elucidated *via* synthesis[40,46-48] and structure-activity studies[14,49,50] of muscimol analogues with different aminoalkyl chains as exemplified in Scheme 2. All of these zwitterions are structural analogues of muscimol and GABA, the charged centres of the compounds being separated by four carbon atoms. Nevertheless, even minor changes of the side chain of muscimol have dramatic effects on the binding of these compounds to the GABA receptors *in vitro*. The affinity of (*RS*)-5-(1-aminoethyl)-3-isoxazolol (IC_{50} 2 μM) and of 4-methylmuscimol (IC_{50} 26 μM) are much lower than that of muscimol (IC_{50} 0.006 μM), and the remaining compounds illustrated in Scheme 2 are virtually inactive. Accordingly, (*RS*)-5-(1-aminoethyl)-3-isoxazolol and 4-methylmuscimol have moderately potent and weak intrinsic activity, respectively, at the GABA receptors *in vivo*. With the exception of (*RS*)-5-(1-aminopropyl)-3-isoxazolol, which is a relatively weak BMC-insensitive depressant of neuronal firing, the rest of the compounds depicted in Scheme 2 are inactive *in vivo*.

Direct steric hindrance may prevent the alkylated muscimol analogues illustrated in Scheme 2 from binding to the receptors with an affinity comparable with that of the parent compound. Alternatively, the alkyl groups may increase the energy of the "receptor-active conformation" compared with that of muscimol, thus preventing these analogues from binding effectively to the GABA receptors. "Active conformations" of flexible and even semirigid



Scheme 2.

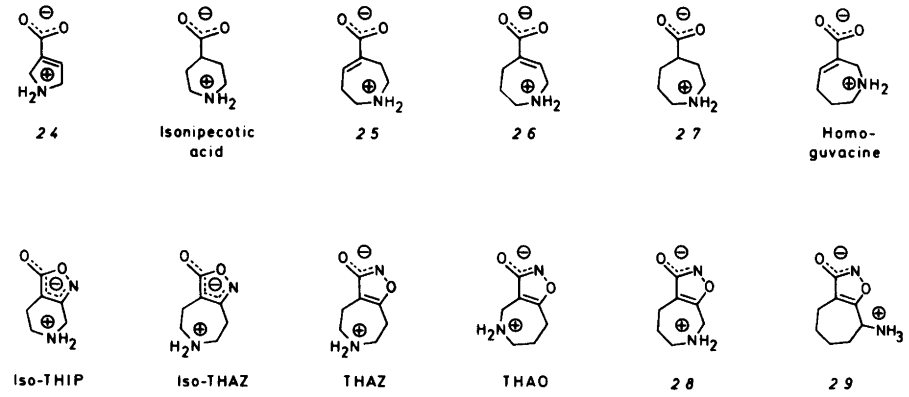


Scheme 3.

biologically active molecules are not necessarily identical with their low-energy conformation(s). Indeed, the conformation of the "muscimol structure element" of the rigid compound THIP (Scheme 3), which is a potent and specific GABA agonist,[10,51] is different from the low-energy conformations of muscimol elucidated by X-ray crystallographic techniques[43] and molecular orbital calculations.[52]

THIP, isoguvacine, and related GABA agonists

The preparation of muscimol analogues, in which the amino functions are incorporated into additional ring structures is an approach to the development of GABA agonists resistent to decomposition *in vivo*. Scheme 3 outlines the syntheses of some of these muscimol analogues and some structurally related amino acids, in which the heterocyclic anionic moieties have been replaced by carboxylate and sulphonate groups. Compound *18*, which is obtained by stereospecific catalytic reduction of *19*, is a common intermediate in the syntheses of isoguvacine and (3*RS*,4*RS*)-3-hydroxyisonipecotic acid (*cis*-3-OH-isonipecotic acid).[53] Similarly the syntheses of THIP (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol),[54] aza-THIP (4,5,6,7-tetrahydropyrazolo[3,4-*c*]pyridin-3-ol)[53] and thio-THIP (4,5,6,7-tetrahydroisothiazolo-[5,4-*c*]pyridin-3-ol) (H. Mikkelsen and P. Krogsgaard-Larsen, unpublished) are based on *19*. Conversion of the ketalized hydroxamic acid, prepared from *19*, into *20* requires much more drastic acid treatment than required for similar cyclization reactions in the syntheses of THPO (4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol)[55] (Scheme 6), THAZ (5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*d*]-azepin-3-ol),[55] THAO (5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*c*]azepin-3-ol),[54] and compound *28*[54] (Scheme 4), probably reflecting a considerable strain in the ring systems of *20* and of THIP. The key problem in the synthesis of thio-THIP is the two-step conversion of the enamine amide *22* into *23*. By analogy with the synthesis of thiomuscimol[39] (Scheme 1) treatment of the enamine intermediate



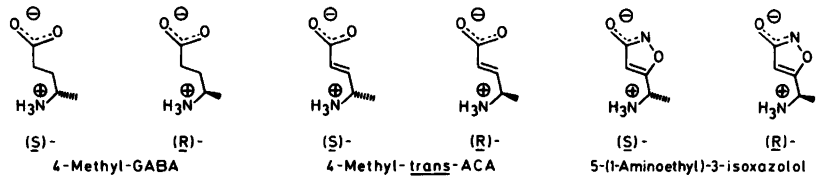
Scheme 4.

(*22*) with hydrogen sulfide did not take the expected course. The reaction product, which upon oxidation gave protected thio-THIP (*23*), appears to be a dimeric compound containing a disulfide bridge.

The conformational mobility of muscimol has been considerably decreased by incorporating the aminomethyl side chain into a second ring structure to give THIP (Scheme 3). The accessible conformations of THIP are within a very narrow range. Since THIP is a potent GABA agonist *in vitro* (IC_{50} 0.1 μM)[10] and *in vivo*,[10,51] with no significant affinity for the enzyme and transport systems in GABA-mediated synapses[10] (Fig. 1), THIP most likely reflects the "receptor-active conformation" of muscimol.[22,23] Structure-activity studies on the bicyclic 3-isoxazolol zwitterions depicted in Scheme 4 emphasize the structural specificity of the GABA receptors.[49] The receptor affinity of these THIP analogues are more than two orders of magnitude lower than that of THIP.

The specificity of the GABA receptors with respect to the structure of the hydroxylated rings of bicyclic muscimol analogues (Schemes 3 and 4), is even more pronounced than that observed for the corresponding monocyclic analogues (Scheme 1). Thus, aza-THIP is completely devoid of affinity for the GABA receptors,[53] and thio-THIP is approximately three orders of magnitude weaker than THIP (H. Mikkelsen and P. Krogsgaard-Larsen, unpublished). While the 5-isoxazolol analogue of muscimol, isomuscimol (Scheme 1) is a very weak GABA agonist,[10] the 5-isoxazolol analogues of THIP, iso-THIP (4,5,6,7-tetrahydroisoxazolo[3,4-*c*]pyridin-3-ol)[53] and iso-THAZ (5,6,7,8-tetrahydro-4*H*-isoxazolo[3,4-*d*]-azepin-3-ol)[56] (Scheme 4), have GABA antagonistic properties.[57]

In accordance with the structural similarity of the 3-isoxazolol anion with the carboxylate group, isoguvacine[49,53] (Scheme 3), which is the amino acid analogue of THIP, is a specific GABA agonist *in vitro* (IC_{50} 0.04 μM)[10] and even more potent than THIP and GABA *in vivo*.[10,51] The saturated analogue of isoguvacine, isonipecotic acid (IC_{50} 0.3 μM) (Scheme 4), and *cis*-3-OH-isonipecotic acid (IC_{50} 12 μM) (Scheme 3) are much weaker than isoguvacine.[51,53] The structure-activity analysis of these cyclic amino acids, which have similar protolytic properties,[53] indicates that GABA agonists with a planar structure in the vicinity of the carboxylate group most effectively bind to and activate the GABA receptors. In the light of these findings it is surprising that piperidine-4-sulphonic acid (P4S)[58] (IC_{50} 0.03 μM) (Scheme 3) is equipotent with isoguvacine as a GABA agonist *in vitro* and *in vivo*.[58] P4S is, however, the only GABA agonist so far known which inhibits the binding of benzodiazepines to the GABA receptor complex, suggesting that the mechanism of interaction of P4S with the GABA receptor is somewhat different from that of other GABA agonists.[59–61] The rigorous structural demands of the GABA receptors on agonists also apply to the isoguvacine analogues *24* (IC_{50} 2 μM),[15] *25* (IC_{50} 15 μM), and *26* (IC_{50} 23 μM),[53] which have much lower affinity for the GABA receptors than isoquvacine (IC_{50} 0.04 μM).[10] Compound *27* and homoguvacine (Scheme 4) are virtually inactive.[53]



Scheme 5.

Stereostructure-activity studies on GABA agonists

The strict structural requirements, which have to be met by GABA agonists, probably reflect that GABA itself binds very tightly to the receptor macromolecule(s). All parts of the molecule of GABA may be in close contact with the receptor, and under these circumstances the achiral molecule of GABA is locked in the "receptor-active conformation" with defined stereochemical orientation. Consequently, the enantiomers of chiral GABA agonists are expected to interact with the GABA receptors in a stereoselective manner. These aspects have been studied in some detail using the synthetic *S*- and *R*-forms of 4-methyl-GABA (P. Krogsgaard-Larsen, unpublished), 4-methyl-4-amino-*trans*-crotonic acid (4-methyl-*trans*-ACA)[62] and 5-(1-aminoethyl)-3-isoxazolol[40] (Scheme 5).

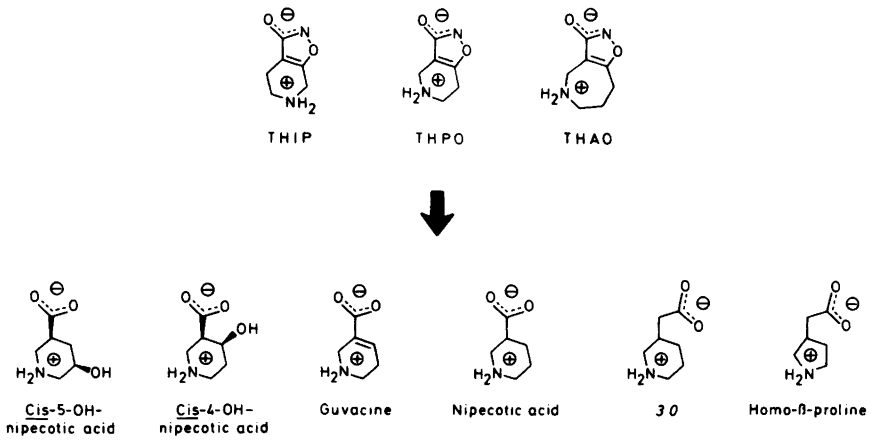
While the *S*-form (IC_{50} 5 μM) and *R*-form (IC_{50} 5 μM) of 4-methyl-GABA have almost the same affinity for the GABA receptors, the *S*-forms of 4-methyl-*trans*-ACA (IC_{50} 4 μM) and of 5-(1-aminoethyl)-3-isoxazolol (IC_{50} 0.6 μM) are approximately thirty times more potent than the respective *R*-forms (IC_{50} 148 and 19 μM).[23,60] These chiral derivatives bind much less effectively to the GABA receptors than the respective unmethylated compounds GABA (IC_{50} 0.03 μM),[10] 4-amino-*trans*-crotonic acid (IC_{50} 0.1 μM)[63] and muscimol (IC_{50} 0.006 μM),[10] indicating that the methyl groups cause steric hindrance for interaction with the receptor. It may be relatively easy for the flexible molecule of 4-methyl-GABA to relieve the steric hindrance caused by the methyl group during the receptor binding, which may explain the equipotency of the *S*- and *R*-forms of this compound. In the case of the more rigid compounds 4-methyl-*trans*-ACA and 5-(1-aminoethyl)-3-isoxazolol, it apparently is more difficult to avoid the unfavourable steric effects of the methyl groups in the *R*-enantiomers at the receptor sites.

The GABA transport carriers also bind chiral inhibitors selectively, but the stereoselectivity of these recognition sites are different from and in some cases opposite to that of the GABA receptors.[15,50] Thus, (*R*)-4-methyl-GABA is a more potent inhibitor of neuronal and glial GABA uptake than the *S*-form. While (*S*)-4-methyl-*trans*-ACA does not interfere with these functions, the *R*-form is a moderately strong inhibitor, in particular of the neuronal system.[15] These results, supported by extensive comparative *in vitro* studies on the GABA receptors and GABA uptake,[14,15,22,50,58,64] suggest that GABA binds to the transport carriers in a conformation different from its "receptor-active conformation".

DESIGN AND DEVELOPMENT OF SPECIFIC GABA UPTAKE INHIBITORS

While THIP is a potent and specific GABA agonist, the isomeric muscimol analogue THPO[55] has very little effect on the GABA receptors.[14,26,49] However, in contrast to THIP, THPO inhibits GABA uptake,[65] interacting selectively with the glial GABA transport carrier, but THPO is only a relatively weak inhibitor (IC_{50} 300 μM).[26,66] The ring-homologue THAO[54] (Scheme 6) has similar effects (IC_{50} 500 μM), but it is completely devoid of receptor affinity.[26,49,66,67]

Following a strategy analogous with that which led to the GABA agonists isoguvacine and related compounds (Scheme 3 and 4), the amino acids related to THPO, namely guvacine[68] and nipecotic acid[69] (Scheme 6), were studied as inhibitors of GABA uptake.[15,65,70] Guvacine and nipecotic acid proved to be very potent on the transport of GABA, being almost equally effective as inhibitors of neuronal (IC_{50} 100 and 70 μM, respectively) and glial uptake (IC_{50} 25 and 30 μM, respectively) (Fig. 1). While isoguvacine and isonipecotic acid are GABA agonists with no effect on GABA transport,[10,26,51] guvacine and nipecotic acid bind



Scheme 6.

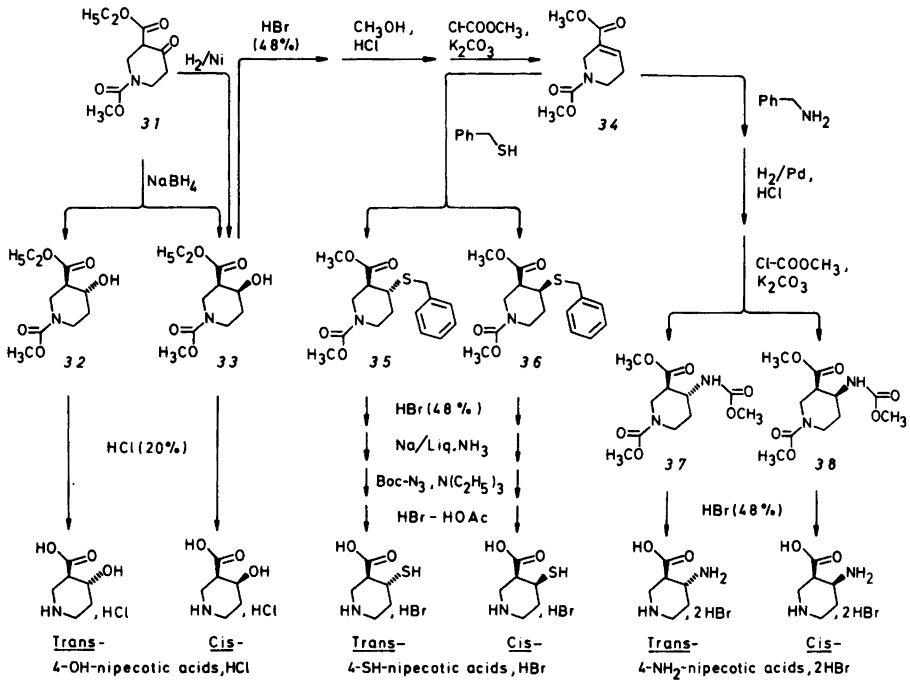
to the transport carriers without interfering with the GABA receptors,[15,26] emphasizing the closely related but distinctly different substrate specificities of these synaptic functions.[22] It is surprising that nipecotic acid, which is not a GABA analogue in the proper sense of the word, is a substrate for the neuronal[71] and also for the peripheral[72] and central[73] glial transport carriers. Furthermore, the affinity of nipecotic acid for the neuronal system is higher than that of GABA itself.[71,74] Kinetic studies suggest that guvacine is also a substrate for the neuronal GABA transport system.[70]

In contrast to nipecotic acid, piperidin-3-ylacetic acid (*30*), which contains a GABA structure element (Scheme 6), has only very little effect on GABA uptake.[63] On the other hand, pyrrolidin-3-ylacetic acid (homo-β-proline) (Scheme 6) is equipotent with nipecotic acid as a neuronal (IC_{50} 75 μM) and a glial (IC_{50} 20 μM) uptake inhibitor.[75] Furthermore, homo-β-proline binds to the GABA receptors with an affinity (IC_{50} 0.2 μM) higher than that of isonipecotic acid (IC_{50} 0.3 μM).[75]

While (3*RS*,5*SR*)-5-hydroxynipecotic acid (*cis*-5-OH-nipecotic acid) has no effect on GABA uptake,[76] the isomeric (3*RS*,4*SR*)-4-hydroxynipecotic acid (*cis*-4-OH-nipecotic acid) (Scheme 6) is the most potent and selective glial uptake inhibitor so far known (IC_{50} 10 μM).[67] Furthermore, *cis*-4-OH-nipecotic acid interferes with the neuronal GABA uptake system (IC_{50} 200 μM),[15,67] and the results of kinetic experiments suggest that it is also a substrate for this transport carrier.[22,26] These observations prompted us to synthesize and test the geometrical isomers of the 4-substituted nipecotic acid analogues illustrated in Scheme 7.

While catalytic reduction of the cyclic β-oxoester *31* gives the *cis*-β-hydroxy ester *33*,[68] sodium borohydride reduction of *31* gives a separable mixture of *32* and *33* (I. Labouta, H. Hjeds and P. Krogsgaard-Larsen, unpublished). (3*RS*,4*SR*)- and (3*RS*,4*RS*)-4-Mercaptonipecotic acid (*trans- and cis*-4-SH-nipecotic acid, respectively) were prepared *via* nucleophilic addition of phenylmethanethiol to the guvacine derivative *34*.[77] The intermediates *35* and *36* were separated chromatographically and deprotected by acid hydrolysis and subsequent debenzylation. However, attempts to isolate the desired mercaptoamino acids directly from the reaction mixtures were unsuccessful, apparently owing to rapid formation of disulfides. The hydrobromides of *trans*- and *cis*-4-SH-nipecotic acid were isolated after introduction of Boc groups on the amino and mercapto groups followed by acid deprotection.[77] The preparation of (3*RS*,4*RS*)- and (3*RS*,4*SR*)-4-aminonipecotic acid (*trans*- and *cis*-4-NH_2-nipecotic acid, respectively)[78] are shown in Scheme 7. Addition of benzylamine to *34* gave an inseparable mixture of products, which were converted into *37* and *38*. Separation of these intermediates by column chromatography followed by acid deprotection gave the desired basic amino acids as dihydrobromides.

The structural constraints imposed on GABA transport inhibitors/substrates are underlined by structure-activity studies of these 4-substituted nipecotic acid analogues. While *cis*-4-SH-nipecotic



Scheme 7.

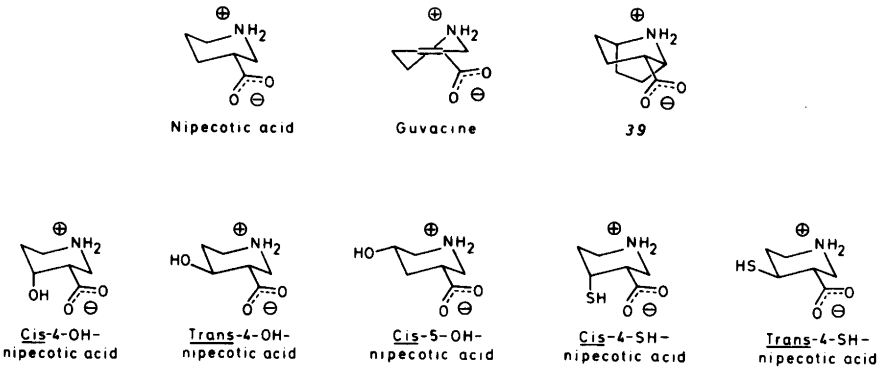
acid is about 15 times weaker than *cis*-4-OH-nipecotic acid as a neuronal GABA uptake inhibitor, *trans*-4-SH-nipecotic acid is inactive.[77] *trans*- and *cis*-4-NH_2-Nipecotic acid[15] and also (3*RS*,4*RS*)-4-hydroxynipecotic acid (*trans*-4-OH-nipecotic acid) (P. Krogsgaard-Larsen and A. Schousboe, unpublished) are inactive with respect to both transport systems.

CONFORMATIONAL STUDIES ON NIPECOTIC ACID ANALOGUES

As part of our attempts to elucidate the structural parameters which have to be considered in the design of potent and specific inhibitors of the GABA transport processes, the conformations of a number of nipecotic acid analogues have been studied. The preferred conformations of these amino acids in aqueous solution and in the crystalline state have been studied using 270 MHz ^{1}H NMR spectroscopic and X-ray crystallographic techniques.

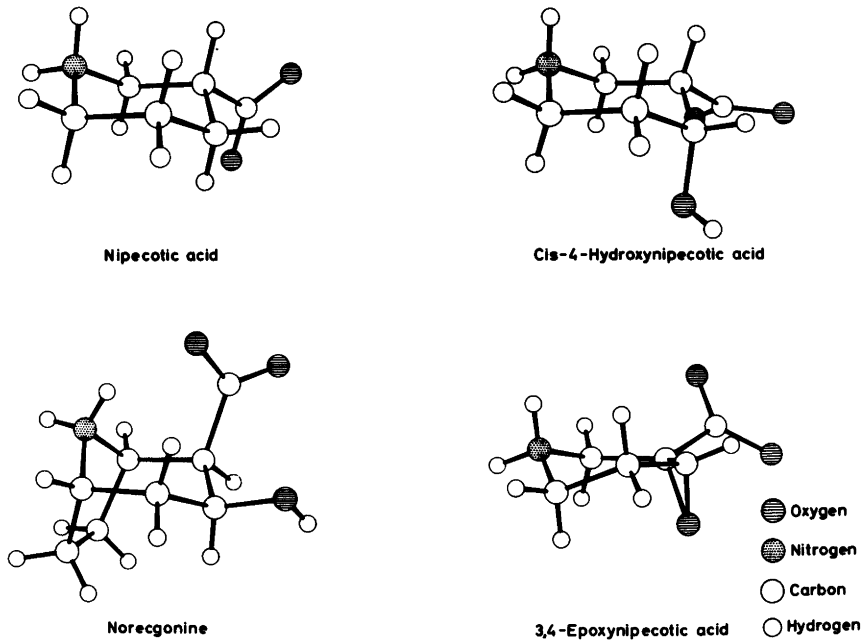
Guvacine and nipecotic acid are equipotent as GABA uptake inhibitors[15,26] (Scheme 8). Although the preferred conformations of the piperidinium rings in nipecotic acid and (1*R*,2*R*,5*R*)-nortropane-2-carboxylic acid (*39*) and the orientations of the carboxylate groups in these compounds are very similar as established by X-ray[79] and/or ^{1}H NMR experiments,[68,79] the latter compound does not affect GABA uptake.[15,26] This lack of activity of *39* indicates that the introduction of an ethylene bridge into nipecotic acid is not compatible with binding to the GABA transport carriers. As already mentioned, the 3-isoxazolol anion in THPO can be considered a rigid carboxylate group. The weak effect of THPO, which has an approximately planar structure, on GABA uptake compared with those of guvacine and nipecotic acid suggests that a certain degree of rotational mobility of the carboxylate groups of the last-mentioned compounds may be important for their biological activities.

The piperidinium rings of *cis*-4-OH-nipecotic acid[80] and nipecotic acid[79] adopt chair conformations, and in both compounds the carboxylate groups have predominantly equatorial orientation. Consequently, the hydroxy group in the former compound is in an axial position.[80]



Scheme 8.

Similarly, ^{1}H NMR spectroscopy has disclosed equatorial and axial orientation, respectively, of the carboxyl and mercapto groups in the hydrobromide of *cis*-4-SH-nipecotic acid,[77] and although *cis*-4-SH-nipecotic acid is much weaker than *cis*-4-OH-nipecotic acid, both compounds bind to the GABA transport carrier. *cis*-5-OH-Nipecotic acid (K. Schaumburg, unpublished) and *trans*-4-SH-nipecotic acid[77] exist predominantly in the conformation illustrated in Scheme 8. Neither compound has any effect on GABA uptake.[76,77] ^{1}H NMR studies of *trans*-4-OH-nipecotic acid (Scheme 8) reveal predominantly diequatorial orientation of the hydroxy and carboxylate groups (K. Schaumburg, unpublished), and *trans*-4-OH-nipecotic acid has no significant effect on neuronal and glial GABA uptake (P. Krogsgaard-Larsen and A. Schousboe, unpublished). These studies indicate that equatorial orientations of the hydroxy and mercapto groups prevent these compounds from



Scheme 9.

binding to the GABA transport carriers.

In the crystals (Scheme 9) nipecotic acid [79] and *cis*-4-OH-nipecotic acid [80] exist in conformations very similar to those observed in aqueous solutions (Scheme 8), emphasizing that these forms of the molecules represent low-energy conformations. The GABA transport carriers may recognize these conformations of the compounds, but the present studies have not shed any light on the conformations, in which nipecotic acid and *cis*-4-OH-nipecotic acid are transported through the synaptic membranes.

As expected from the above discussion, norecgonine (Scheme 9), which has an ethylene bridge similar to that in the inactive compound *39* (Scheme 8), has no effect on GABA uptake.[15] Furthermore, the hydroxy and carboxylate groups in norecgonine have equatorial and axial orientations in the crystals and in aqueous solutions, which is opposite to the preferred orientations of these groups in *cis*-4-OH-nipecotic acid (L. Brehm and K. Schaumburg, unpublished).

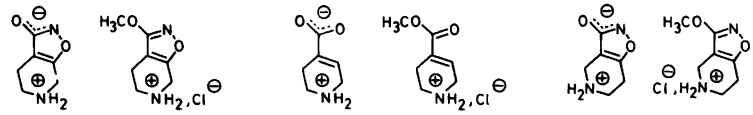
(3*RS*,4*SR*)-3,4-Epoxynipecotic acid (3,4-epoxynipecotic acid) (Scheme 9), which like *cis*- and *trans*-4-SH-nipecotic acid was designed as an inhibitor with irreversible binding to the GABA transport carriers, is almost two orders of magnitude weaker than guvacine and *cis*-4-OH-nipecotic acid.[77] Like the double bond in guvacine, the epoxy group in 3,4-epoxynipecotic acid makes the surroundings of the carboxylate group conformationally restrained. However, since guvacine is a very active inhibitor this molecular rigidity of 3,4-epoxynipecotic acid alone can hardly explain its low affinity for the GABA transport carrier. Steric effects of the epoxy group evidently have to be considered, and although the orientation of this group is similar to the preferred orientation of the hydroxy group in *cis*-4-OH-nipecotic acid (Schemes 8 and 9), greater conformational mobility may allow the latter compound to adopt a conformation, which fits the binding site of the carrier molecules.

PHARMACOLOGICAL AND THERAPEUTICAL ASPECTS

The present studies, initiated by structural manipulations of the natural product muscimol, have disclosed a high degree of structural specificity of the GABA synaptic processes (Fig. 1), and they have shed some light on the structural parameters of importance for GABA agonists and uptake inhibitors. However, the therapeutical interest in these types of compounds demands consideration also of toxicological and pharmacokinetic properties. Thus, a necessary condition for studies in man is that the compounds are capable of entering the central nervous system after peripheral administration. GABA does not penetrate the BBB,[21] and similarly the amino acids isoguvacine [81] and nipecotic acid [82] do not enter the brain after systemic administration.

In contrast to muscimol, THIP has a very low toxicity in animals,[25] and THIP is resistent to metabolic decomposition in humans and animals, being excreted unchanged and partially conjugated in the urine.[81] THIP is at present the subject of comprehensive clinical studies.

In spite of the zwitterionic structures of both THIP and isoguvacine (Scheme 10) THIP, but not the latter compound, is capable of penetrating the lipophilic BBB,[81] and THPO also affects GABA neurones after peripheral administration.[83] These differences probably can be explained exclusively on the basis of the different protolytic properties of the compounds (Scheme 10). The pK_A values of isoguvacine are considerably different from those of



	THIP	O-Methyl-THIP, HCl	Isoguvacine	Isoguvacine methyl ester, HCl	THPO	O-Methyl-THPO, HCl
pK_A values	4.4; 8.5	7.1	3.6; 9.8	8.9	4.3; 9.1	7.7
[zwitterion] / [unionized mol.]	500		200000		2500	

Scheme 10.

THIP and THPO. Using these values and the pK_A values for the corresponding O-methyl derivatives, Wegscheider's method [84] allows a calculation of the ratios between the zwitterionic and unionized forms of these compounds in aqueous solutions. As illustrated in Scheme 10 this ratio is strictly dependent on the pK_A values.[81] Thus, an aqueous solution of THIP contains approximately 0.2 % of unionized THIP, and since all these compounds probably have to pass the BBB in the unionized forms, it is understandable that THIP and THPO enter the brain from the blood stream much more readily than isoguvacine.

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